biology and life sciences forum

MDPI

*Abstract*

# Evaluating Repetitive Mucus Extraction Effects on Mucus Biomarkers, Mucus Cells and Skin-Barrier Status in a Marine Fish Model †

**Ignasi Sanahuja [1,2,*], Pedro M. Guerreiro [3,‡], Albert Girons [4], Laura Fernández-Alacid [1] and Antoni Ibarz [1]**

[1] Department of Cell Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona, Avda. Diagonal 643, 08028 Barcelona, Spain; fernandez_alacid@ub.edu (L.F.-A.); tibarz@ub.edu (A.I.)
[2] Aquaculture Program, Institute of Agrifood Research and Technology (IRTA), 43540 La Ràpita, Spain
[3] CCMAR-Centre for Marine Sciences, University of Algarve, 8005-139 Faro, Portugal; pmgg@ualg.pt
[4] Ictiovet-Veterinary Services for the Aquaculture, 08025 Barcelona, Spain; albert.girons@ictiovet.com
* Correspondence: isanahuja@ub.edu
† Presented at the IX Iberian Congress of Ichthyology, Porto, Portugal, 20–23 June 2022.
‡ Presenting author (poster presentation).

**Abstract:** Among all the mucosal barriers, the skin and its surrounding mucus are possibly the main defensive tools used by fish against the environment. Due to its less-invasive extraction, the study of its production and functions has gained high interest in the last years. However, there are still many gaps in research, such as the possible alteration of mucus composition or the skin integrity resulting from the sampling process. In the current study, skin mucus extraction impacts were determined by comparing the effects of one-single extraction (SEG; single extraction group) and three successive extractions (REG; repetitive extractions group, separated by 4 days) on mucus properties and on skin epithelial integrity. In terms of analytical evaluation, plasma biomarkers and plasma antibacterial capacity were also determined. With regard skin histology and skin barrier properties, both SEG and REG did not evidence differences with respect to intact skin (ØEG). Interestingly, the repetitive mucus extraction protocol seemed to activate skin mucus turnover, significantly increasing the number of low-size mucus cells (cell area < 100 $\mu m^2$) and reducing the number of high-size mucus cells (cell area > 150 $\mu m^2$). Repetitive extraction of skin mucus diminished the amounts of soluble protein and glucose in mucus with regard to one-single extraction and increased cortisol exudation. These metabolites remained unaltered in plasma, indicating the different response among both sampling targets. Despite mucus biomarkers modification, the antibacterial capacity against the pathogenic bacterial (*P. anguilliseptica* and *V. anguillarum*) was maintained in both plasma and mucus irrespective of the number of mucus extractions. Overall, the mucus sampling protocol scarcely affected skin integrity and mucus antibacterial properties and only modified metabolites exudation, evidencing a feasible and minimally invasive method for studying fish health and welfare as an alternative or as a complement to plasma. The knolwdege provided here highlighted that this methodology is putatively transferable to farm culture conditions and showed that it is very useful for the study of threatened species aimed at preserving fish welfare.

**Keywords:** skin mucus; mucus barrier; biomarkers; antibacterial activity



**Citation:** Sanahuja, I.; Guerreiro, P.M.; Girons, A.; Fernández-Alacid, L.; Ibarz, A. Evaluating Repetitive Mucus Extraction Effects on Mucus Biomarkers, Mucus Cells and Skin-Barrier Status in a Marine Fish Model. *Biol. Life Sci. Forum* **2022**, *13*, 96. https://doi.org/10.3390/blsf2022013096

Academic Editor: Alberto Teodorico Correia

Published: 15 June 2022



**Author Contributions:** Conceptualization, I.S., P.M.G., L.F.-A. and A.I.; methodology, I.S., P.M.G., L.F.-A. and A.G.; validation, I.S., P.M.G., L.F.-A. and A.I.; formal analysis, I.S., P.M.G., L.F.-A. and A.I.; investigation, I.S., P.M.G., L.F.-A. and A.I.; resources, P.M.G. and A.I.; data curation, I.S. and L.F.-A.; writing—original draft preparation, I.S.; writing—review and editing, I.S., P.M.G., L.F.-A. and A.I.; funding acquisition, A.I. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by means of grants from the Spanish Government: PID2019-106878RB-I00 and IS was granted with a Postdoctoral fellowship (FJC2020-043933-I).

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the European Union Council (Directive 2010/63/EU) and Portuguese legislation for the use of laboratory animals (Decreto-Lei n.º 113/2013 de 7 de Agosto), and approved by the Institutional Review Board of CCMAR, Adelino V. M. Canário, on 3 April 2021.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.